Microbial Contamination in Inoculated Shell Eggs: II. Effects of Layer Strain and Egg Storage

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ABSTRACT Three Ottawa control strains and a current commercial laying stock were reared and housed in the same environment. Eggs were collected at 5 different hen ages throughout the 2 production cycles of the flock. The eggs were inoculated with *Salmonella* Enteritidis (SE), *Pseudomonas fluorescens* (PF), a combination of the 2, or sterile buffered peptone water and stored up to 5 wk. After storage at room temperature, contamination levels were determined for the exterior surface, air cell, egg contents, and within the shell. Interior, egg contents, and shell contamination levels of SE and PF increased with storage time. There were no apparent increases in the infectivity of SE or PF in the presence of the other organism. PF was a poor survivor on the shell surface under these storage conditions. Throughout the 5-wk storage, eggs from control strain 10 maintained their microbial integrity more effectively. Eggs from control strain 5 and the current commercial stock were more easily contaminated than the other strains. These data suggest that genetic selection has altered microbiological defenses of the eggs produced.

(*Key words*: genetics, *Pseudomonas fluorescens*, *Salmonella* Enteritidis, shell egg, spoilage)

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INTRODUCTION

Recent identification of numerous emerging pathogens in the United States has caused increased consumer awareness of food safety issues. *Salmonella* Enteritidis (SE) has been identified as one of these emerging pathogens. SE has often been associated with raw or undercooked shell eggs and egg products, among other foods. Due to these concerns, public perception of a "good egg" has shifted from shell cleanliness and physical properties to that of microbial integrity.

In the past, genetic selection of laying hens has focused primarily on production qualities and traits such as egg size, egg output, and feed conversion. Microbial integrity has not been a priority because much of selection has been consumer driven. Food safety concerns associated with shell eggs did not gain importance until the early 1990s. Lorenz et al. (1952) reported that different birds produced eggs with different infection rates of *Pseudomonas* when inoculated with a common inoculum. Also, Trussell et al. (1955) found that the breed of a hen had a direct effect on penetration of spoilage organisms after processing. Furthermore, Stokes et al. (1956) identified several factors that can affect the ability of *Salmonella* to penetrate an egg. Many of these factors such as porosity of the shell, thickness of shell membrane, and concentration of natural antimicrobials could be altered by genetic selection.

A majority of the work conducted in the past has encompassed egg safety and cleanliness. These data can be difficult to compare due to differences in rearing and management practices utilized for laying hens. There have been changes in the methods of storing eggs. In the 1950s and 1960s, before photostimulation and lighting programs were used to enhance egg production, it was common to store eggs in excess of 60 d to ensure a constant supply of eggs. Currently most eggs processed in the United States have an average sell by date of 30 d and are sold by 19 d postprocessing (Bell et al., 2001; Patterson, et al., 2001).

The current study was designed to compare the eggs produced from different closed, random-bred strains of commercial laying hens that were reared and managed under the same environmental conditions. These eggs were compared to see how they would withstand a sur-

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Abbreviation Key: CCS = current commercial stock; CS5 = control strain 5; CS7 = control strain 7; CS10 = control strain 10; MAC+NA = MacConkey agar with nalidixic acid; PF = *Pseudomonas fluorescens*; PIA = *Pseudomonas* isolation agar.

face microbial challenge. SE and *Pseudomonas fluorescens* (PF) were used to inoculate the eggs. Both of these organisms are gram negative, which Board (1966) reported are more capable of withstanding antimicrobials present in the albumen. SE was selected because it is the primary pathogen associated with shell eggs. PF has been identified as a spoilage organism associated with shell eggs (Banwart, 1989). It has also been previously identified as a primary invader of the shell membranes and predicted to allow other organisms to better traverse the membranes (Florian and Trussell, 1957). Humphrey et al. (1991) have also found that egg age has an effect on SE growth. The eggs in this study were stored for a period over the average 30 d sell by date found in the United States to explore the changes that occur.

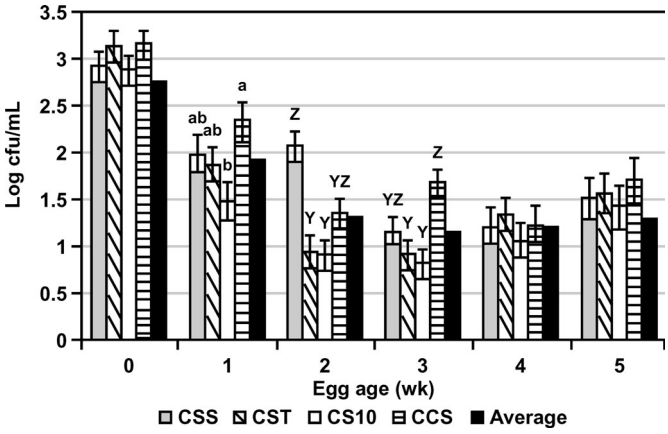

FIGURE 1. Effect of egg age and hen genetic strain on exterior *Salmonella* Enteritidis contamination of shell egg. [ab]Means with similar letters are not different at $P < 0.05$. [YZ]Means with similar letters are not different at $P < 0.0001$.

MATERIALS AND METHODS

Source of Shell Eggs

Eggs were collected from 4 genetic stocks during this study. Three Ottawa Control Strains, provided by Agriculture Canada, and a current commercial laying strain were all hatched and reared under the same environmental and management conditions at the North Carolina Department of Agriculture and Consumer Services Piedmont Research Station, Salisbury, North Carolina. The random-bred control strains utilized were strains 5 (CS5), 7 (CS7), and 10 (CS10) as described by Gowe et al. (1993) and Fairfull et al. (1983). Selection was closed in these strains in 1950, 1959, and 1972, respectively. The current commercial stock was a 1993 laying stock with a common ancestral linkage to the control strains. Molt was induced in the complete flock at 62 wk of age. The first production cycle peak occurred from 28 to 32 wk of age. The second production cycle peak occurred at 68 wk of age (Jones et al., 2001). Large eggs were collected at hen ages 32, 45, 58, 71, and 84 wk. Hen age effects were reported in a previous publication (Jones et al., 2002).

Inoculation Technique

The standard inoculation technique (Brant et al., 1965) was used for nalidixic acid resistant SE and PF inoculation procedures as described in Jones et al. (2002). Eggs were inoculated at 10^6 cfu / mL for each organism and stored in an incubator at 26°C and 90 ± 5% RH. The survival of SE has been reported to be enhanced at higher RH (Lancaster and Crabb, 1953). Furthermore, Gast and Beard (1992) reported that the isolation of SE from eggs stored at room temperature was significantly greater than from eggs stored at 7.2°C for 7 d. Eggs were stored, and 12 from each treatment group was tested once a week for 5 wk.

Experimental Design

Eggs were sampled at 4 locations: external shell rinse, internal air cell, egg contents, and shell contents according to the methods reported by Jones et al. (2002). SE was cultured on MacConkey agar with 200 μL of nalidixic acid / mL added (MAC+NA). These plates were incubated at 35°C for 48 h before colony counts were determined. PF was detected on pseudomonas isolation agar (PIA). All PIA plates were incubated at 26°C for 48 h before enumeration. The noninoculated egg samples and those from eggs inoculated with the combination for SE and PF were plated on MAC+NA and PIA. All other egg samples were plated on the medium appropriate for the organism used in the inoculum.

Statistical Analysis

All data were subjected to log transformation before analysis was conducted. Due to the prominence of 3-way interactions, data were sorted for egg age. Data were analyzed using the general linear model of SAS software (SAS, 1989) with hen age as a block. Means were separated by the least square method. Data from the inoculation combination were not significant for the single organism versus combination inoculum. Therefore, for brevity, these data are not presented in the current manuscript.

RESULTS AND DISCUSSION

Figure 1 illustrates the changes in SE contamination of the exterior of the egg during 5 wk of storage. At time zero, SE levels were not different among the strains. After 1 wk of storage the current commercial stock (CCS) had the highest ($P < 0.05$) degree of contamination as compared with the other strains. These levels were lower than those observed immediately after inoculation. Average SE shell contamination levels decreased until 2 wk of storage when they remained slightly above 1 log cfu / mL for the remainder of the study. Throughout the storage period, CCS maintained a high level of SE contamination on the shell surface. Consequently, CS10 exhibited the lowest level of SE shell contamination at each week of

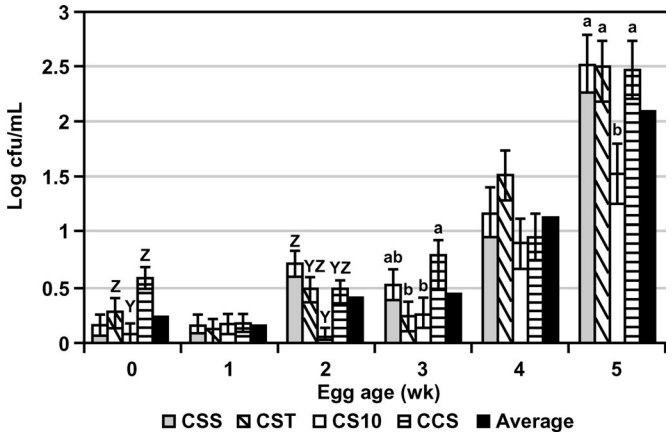

FIGURE 2. Effect of egg age and hen genetic strain on interior *Salmonella* Enteritidis contamination of shell eggs. [a,b]Means with similar letters are not different at $P < 0.05$. [Y,Z]Means with similar letters are not different at $P < 0.001$.

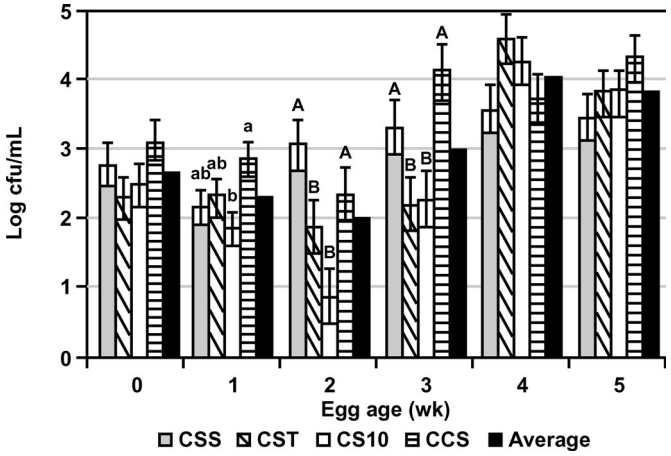

FIGURE 4. Effect of egg age and hen genetic strain on shell *Salmonella* Enteritidis contamination of shell eggs. [AB]Means with similar letters are not different at $P < 0.01$.

storage. These results suggest that the shell structure of the CS10 eggs were less accommodating for the growth of SE.

Average interior air cell SE contamination began to increase each week after 3 wk of storage (Figure 2). When significant differences were found among the strains, CCS always maintained one of the greatest levels of contamination, and CS10 had one of the lowest. At 5 wk, CS10 had 1.5 log cfu / mL present in the air cell compared with an averaged 2.5 log cfu / mL for the 3 other strains.

The contamination of the egg contents with SE increased during egg storage (Figure 3). When significant differences occurred among the strains, CS5 exhibited a higher level of SE contamination, and CS10 maintained one of the lowest levels. The differences among the strains may be due to the effectiveness of the natural antimicrobials present in the albumen of the eggs. At 4 and 5 wk of storage, no differences could be found among the strains. This finding could illustrate an end to the effective period for natural antimicrobials present in the eggs.

Figure 4 illustrates the changes in shell SE contamination during egg storage. Average SE shell contamination levels were lowest at 2 wk of storage (2.1 log cfu / mL). When significant differences were observed among strains (wk 1, 2, and 3), CS5 and CCS had the greatest SE levels present in the shell, and CS10 maintained the lowest levels. Within the genetic strains compared in this study, CS10 appeared to be more capable of resisting SE contamination and growth, whereas CCS had the least capability. SE growth increased in all strains at wk 4 and 5 of storage except on the shell surface. There were few significant differences found among the strains during this period, suggesting an overall decrease in the effectiveness of the natural antimicrobial compounds present in the egg.

The level of exterior PF contamination was greatest immediately after inoculation (Figure 5). After 1 wk of storage, PF growth decreased by greater than 1.5 log cfu / mL. Levels gradually increased throughout the remainder of the storage time with differences occurring at 2 wk (*P*

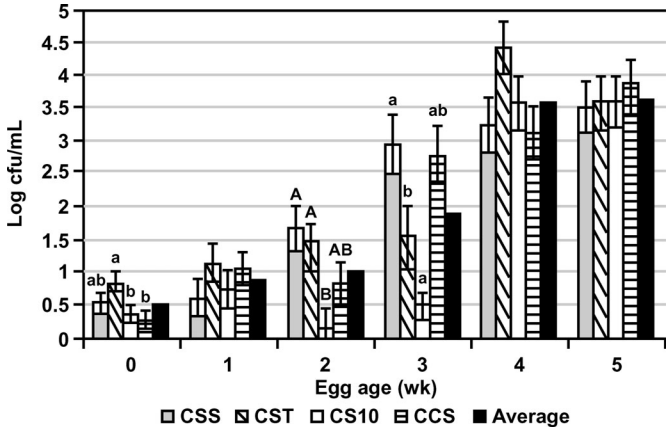

FIGURE 3. Effect of egg age and hen genetic strain on egg contents*Salmonella* Enteritidis contamination of shell eggs. [a,b]Means with similar letters are not different at $P < 0.05$. [A,B]Means with similar letters are not different at $P < 0.01$.

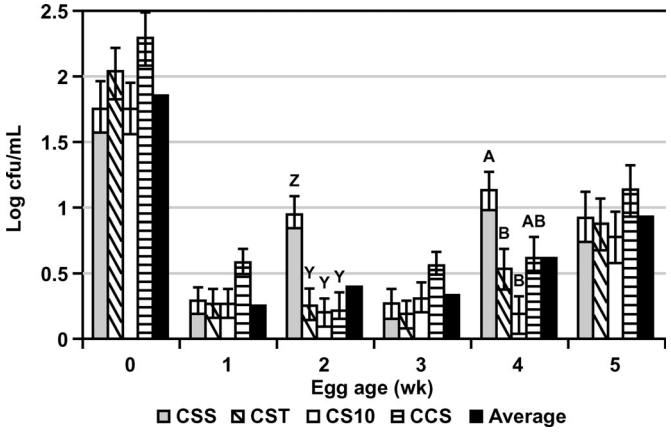

FIGURE 5. Effect of egg age and hen genetic strain on exterior *Pseudomonas fluorescens* contamination of shell eggs. [A,B]Means with similar letters are not different at $P < 0.001$. [Y,Z]Means with similar letters are not different at $P < 0.0001$.

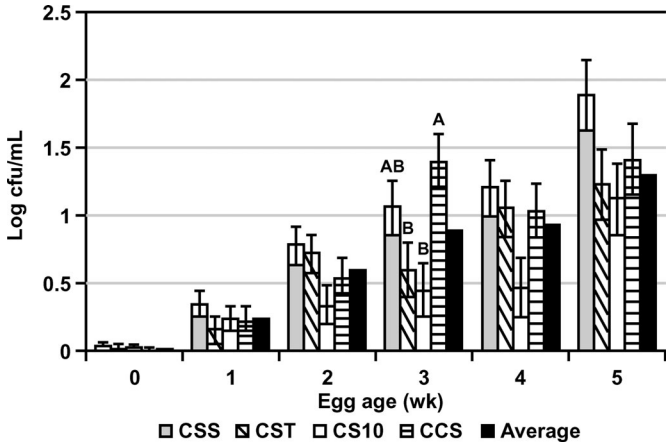

FIGURE 6. Effect of egg age and hen genetic strain on interior *Pseudomonas fluorescens* of shell eggs. [A,B]Means with similar letters are not different at $P < 0.01$.

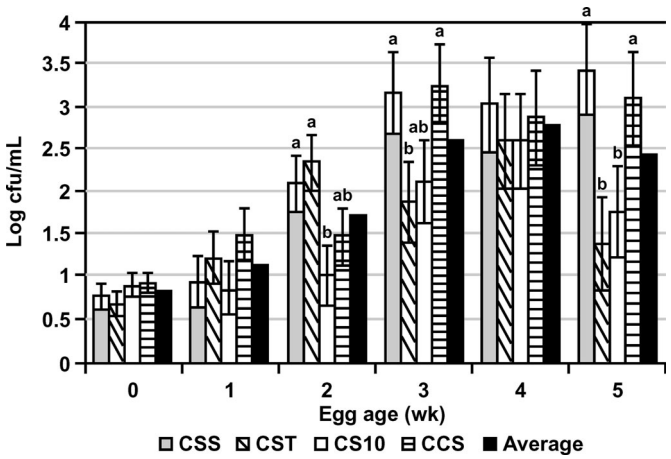

FIGURE 8. Effect of egg age and hen genetic strain on shell *Pseudomonas fluorescens* contamination of shell eggs. [a,b]Means with similar letters are not different at $P < 0.05$.

< 0.0001) and 4 wk ($P < 0.01$) of storage. During both of these periods, CS5 had the greatest PF contamination, and CS10 had the least PF contamination. The sharp initial decrease and subsequent gradual increase in PF levels suggest that current storage conditions were not favorable for optimum PF growth, and after a period of adaptation, some organisms were then able to thrive.

Interior air cell PF contamination increased throughout storage (Figure 6). The genetic strains were different ($P < 0.01$) at 3 wk of storage. CCS and CS5 had a greater level of PF contamination in the air cell at this time. The 3 control strains were not different from each other, but CS10 did maintain the lowest level of contamination. During the other sampling periods, CS10 had one of the lowest levels, if not the lowest, of PF air cell contamination, as was observed with SE populations.

In general, PF contamination of the egg contents increased during the 5 wk of storage (Figure 7). Immediately after inoculation, higher levels ($P < 0.05$) of PF were detected in the contents of CS5 and CS7 eggs. After 2 wk of storage, CS5, CS7, and CCS all had higher levels ($P < 0.01$) of PF contamination present in the contents of eggs. During both of these sampling periods, CS10 maintained the lowest level of PF contamination. Both SE and PF followed similar trends for growth in the egg contents.

Figure 8 shows the gradual increase in shell PF contamination during storage. At 2, 3, and 5 wk of storage, differences ($P < 0.05$) were observed among genetic strains. CCS and CS5 generally had the greatest levels of contamination, and CS10 maintained one of the lowest during these periods. These data indicated that SE contamination of the shell was more aggressive compared with PF. Initially, average SE contamination of the shell was 2.7 log cfu / mL compared with 0.8 log cfu / mL for PF. The greatest average levels of contamination for both organisms occurred at 4 wk of storage with SE having a value 1.3 log cfu / mL greater than PF.

Mean SE and PF counts obtained from various egg components are shown in Figures 9 and 10, respectively. SE survived on the exterior of the eggs tested much longer than did PF. Interior SE counts were < 0.5 log cfu / mL

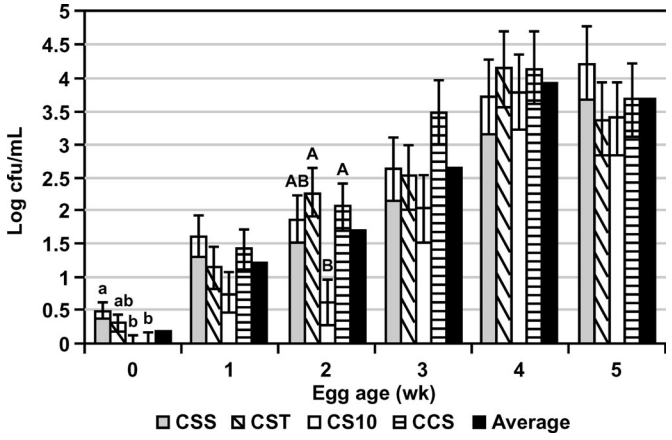

FIGURE 7. Effect of egg age and hen genetic strain on egg contents *Pseudomonas fluorescens* contamination of shell eggs. [a,b]Means with similar letters are not different at $P < 0.05$. [A,B]Means with similar letters are not different at $P < 0.01$.

FIGURE 9. Average *Salmonella* Enteritidis counts obtained from egg components.

FIGURE 10. Average *Pseudomonas fluorescens* counts obtained from egg components.

for 3 wk and then increased dramatically. In contrast, interior PF counts increased steadily throughout the trial. SE counts in egg contents increased about 0.5 log cfu / mL during the first 2 wk of the trial and then increased more rapidly until reaching a plateau of approximately 3.5 log cfu / mL at 5 wk of storage. Egg content PF counts also plateaued at 5 wk, but initial levels were greater than those observed in SE counts. SE shell counts were virtually parallel to egg content results. These data suggest that SE and PF contaminate egg components in different manners.

Previous research has shown that eggs with different shell permeabilities maintained similar spoilage levels until 15 d of storage when the most permeable began to spoil more quickly (Fromm and Monroe, 1960). In the current study, SE contamination of the contents was not different among the strains until after 14 d of storage. These results were not observed for corresponding PF levels. Because permeability of the egg was not monitored in the current work, it cannot be ruled out that selection could have affected this parameter and produced the current findings. Similar to the finding associated with hen age (Jones et al., 2002), CS10 eggs were able to maintain a higher level of microbiological integrity throughout storage compared with the other genetic strains tested. CCS and CS5 had greater levels of microbial contamination as compared with the other strains evaluated during storage. These finding indicate that selection processes did have an effect on microbial integrity, although this trait was not selected for at the time.

Shell membranes have been recognized as the primary physical barrier to microbial infection of the egg contents (Florian and Trussell, 1957; Kraft et al., 1958; Hartung and Stadelman, 1962). Florian and Trussell (1957) reported PF as being able to penetrate the shell membrane within 20 h of inoculation. Board (1966) had reported a 20-d difference between the time organisms penetrated the shell and large numbers of organisms being found in the contents of the egg. The current research found the contents to be contaminated with both SE and PF within 24 h of inoculation. The concentration of inoculum used in the current study and the incubation at room temperature might have led to these discrepancies. Board (1966) suggested that property changes within the shell membrane were responsible for the ability of organisms to more readily infect the egg contents during storage. He was unable to distinguish whether these changes were chemical, physical, or a combination of both. In the current study, storage of the eggs at room temperature might have accelerated the degradation of the antimicrobial systems of the egg.

Humphrey (1994) reported that there was no information that SE could move through the shell membrane at the expense of other organisms. Florian and Trussell (1957) reported that PF served as a primary invader and allowed other organisms to gain access to the egg contents, which otherwise would not have been able to traverse the shell membranes. The current work does not show a preference for SE to move through the membrane, nor do these findings suggest that PF aided SE in penetrating the shell membranes. Garibaldi and Bayne (1962) disputed the classification of primary or secondary invader for microorganisms and stated that environmental conditions play a role in an organism's ability to penetrate the shell and shell membranes.

Inoculation of the air cell with bacteria has resulted in an increased growth rate at room temperature after 4 d (Miller and Crawford, 1953). The current study found similar results for PF growth. Contamination levels present in the air cell progressively increased throughout storage. Comparable findings were not observed for SE growth. SE levels present in the air cell remained at a relatively constant level in the inoculated eggs until 4 wk of storage when the growth rate appears to have increased.

The results of this study show that genetic selection has altered the egg's ability to withstand microbial contamination and penetration during storage. With current consumer concern for food safety, it may be beneficial for breeders to examine microbial integrity of shell eggs as a screening tool to enhance table egg safety and quality.

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